comprising a nucleic acid encoding a polypeptide for treating said disease or disorder, wherein the surface bound growth factor induces the quiescent cells to divide, so that the nucleic acid encoding the polypeptide for treating said disease or disorder can incorporate into the genome of the cells; and

b) administering to the patient an amount of the cells of step (a) effective to treat said patient's disease or disorder.

REMARKS

At the outset, Applicants wish to thank the Examiner for the telephone interview in which it was emphasized that the methods of the invention are distinguished over the prior art because the prior art does not teach the exposure of quiescent target cells to a retroviral packaging cell line. The Examiner also agreed to consider the proposed amendments and a Rule 132 Declaration regarding the likelihood of success of the human treatment claims.

Claims 9 and 10 are canceled. Claim 11 is amended. Claims 1-8 and 11-12 are currently pending.

Rejections under 35 U.S.C. §112, first paragraph:

Claims 10-12 are rejected under 35 U.S.C. §112, first paragraph for alleged lack of enablement. The Office Action states that the method as claimed will transform any cell, not just a quiescent cell, that has the receptor for the growth factor expressed on the surface of the packaging cell line. Applicants submit that the proposed amendment to claim 11, from which claim 12 depends, specifies that *quiescent* cells are exposed to a retroviral packaging cell line. Applicants submit that the proposed amendment is sufficient to overcome this basis of rejection. Applicants note that this limitation does not exclude the presence or transformation of non-quiescent cells in the sample containing a population of quiescent cells to be transformed.

The Office Action also alleges that claims 10-12 are not enabled because, in addition to autologous cells, the claims encompass the administration of allogeneic and xenogeneic cells to a patient, and concludes that the art of such transplantation is unpredictable. Applicants respectfully disagree.

First, as noted in the previous response, the specification acknowledges at page 9, lines 18-24, that in some instances immune rejection of implanted cells may be a problem, and teaches that the expression of Fas ligand on the packaging cell line can prevent such rejection. It was

therefore known in the art at the time of filing that Fas ligand could be used to prevent or limit immune rejection of implanted allogeneic cells. In addition to this, Applicants submit that there is a long history in medicine of immunosuppressive drug regimens and their effect on the rejection of implanted tissues.

The topic of cellular transplantation and strategies of reducing tissue immunoreactivity with a minimun of recipient immunosuppression is covered in the review by Gill & Wolf, July-August 1995, Cell Transplant 4: 361-370 (Exhibit A). The review focuses on the T cell response to allogeneic and xenogeneic transplants and implications of this reactivity on the field of cellular replacement therapy.

A small sample of the numerous prior art references illustrating the knowledge in the art regarding immunosuppression to avoid transplant rejection includes 1) Human allografts: Foster et al., June 1995, Transplantation 59: 1557-1563 (Exhibit B; granulocyte colony stimulating factor in combination with standard immunosuppression improves human liver allograft rejection and sepsis in recipients); Abouna et al., June 1995, Transplantation 59: 1564-1568 (Exhibit C; adjusting the daily dose of antithymocyte globulin based on T cell monitoring avoids overimmunosuppression and reduces cost of therapy following human renal allografts); Suthanthiran & Strom, July 1995, J. Clin. Immunol. 15: 161-167 (Exhibit D; greater than 80% human solid organ graft survival at one year post-implantation, due to immunosuppressive regimes); Fullerton et al., April 1995, Ann. Thorac. Surg. 59: 804-811 (Exhibit E; human cardiac transplantation may be performed with excellent early and intermediate-term results, in which induction therapy with antithymocyte therapy and drug-based immunosuppression plays a large role); Swenson et al., April 1995, J. Am. Coll. Cardiol. 25: 1183-1188 (Exhibit F; switching pediatric heart transplant patients from cyclosporin A to FK506 for immunosuppression has benefits to the patients); and Czech & Sagen, August 1995, Prog. Neurobiol. 46: 507-529 (Exhibit G; cellular transplantation in the CNS for management of chronic pain in both humans and rats); 2) a large number of animal allograft studies, e.g., Li et al., September 27, 1995, Transplantation 60: 523-529 (Exhibit H; showing tolerance induction for avoiding rejection of pancreatic islet cell allografts in mice); and Gianello et al., March 1995, Transplantation 59: 884-890 (Exhibit I; induction of tolerance for renal allografts in miniature swine); Harada et al., September 15, 1995, Transplantation 60: 517-519 (Exhibit J; priming a recipient with donor spleen cells and activated B cells induces prolonged survival of skin allografts in

cyclophosphamide-treated mice); and Nakanishi & Yasumoto, September 1995, Ann. Thorac. Surg. 60: 635-639 (Exhibit K; identification of optimal reduced dosages of cyclosporin A for tracheal allografts in dogs) and 3) xenotransplant studies in animals, e.g., and Nishimura et al., August 1995, Immunobiology 193: 420-438 (Exhibit L; rat-to-mouse skin xenograft tolerance induction)

In view of the widespread and detailed prior knowledge in the art with regard to avoiding immune rejection of donor tissues and cells, Applicants submit that one skilled in the art of tissue transplantation and immunosuppression would have been capable of limiting immune rejection of non-autologous cells transformed according to the invention as of the September 28, 1995 priority date of this application.

Second, the Examiner had expressed concern that an immune response to the retroviral packaging cells would prevent the transfer of the nucleic acid encoding a polypeptide for treating a disease or disorder to the target cells. Applicants submit that claim 11 as amended is limited to cells transformed *in vitro* by exposing quiescent cells to a retroviral packaging cell line expressing nucleic acid encoding a growth factor that is displayed on the surface of the cell line. Thus, claim 11, and claim 12 that depends from it, do not require the avoidance of an immune response that might prevent the transfer of the nucleic acid. Applicants submit that the *in vitro* exposure of the quiescent cells to a packaging cell line avoids any immune problems with regard to the actual transformation of the quiescent cells. Once transformed cells are generated in vitro, the immune issues surrounding their implantation into a patient are the same as those faced in any non-autologous cell transplant situation, and, as described above, at the time the application was filed it was known in the art how to limit the rejection of implanted cells.

The Office Action also states that "there is no evidence that the claimed method works in vivo and produces art accepted levels of a protein in vivo," and that "the specification has not provided any evidence whether quiescent cells in vivo would be transformed by the claimed method in vivo." Applicants submit that amended claim 11 specifically recites the transformation of quiescent cells *in vitro*, before the administration of the transformed cells to a patient. Because the transformation of quiescent cells in the claimed method of treatment occurs in vitro, there is no requirement that the transformation method work in vivo. The specification clearly enables the transformation of quiescent cells in vitro and shows that they produce readily detectable levels of a transduced gene product. See for instance, Examples 1 and 2, which

demonstrate the expression of a β-galactosidase gene by cells transduced according to the method recited in claim 11. See also Example 3, in which quiescent hematopoietic stem cells were transformed with a p47-phox sequence using methods according to the invention.

With regard to the expression of art-accepted or therapeutic levels of a polypeptide for the treatment of a disease or disorder, Applicants submit that quiescent cells transformed in the manner recited in claim 11 as amended are no different, as far as expression is concerned, than cells transduced by methods in the prior art. The distinction of the claimed methods over the prior art is that they allow the efficient transformation of quiescent cells that until now have been refractory to transformation (e.g., stem cells). Once a construct has been successfully introduced to a quiescent cell, there is no reason to believe that that cell will not produce an effective amount of a polypeptide for the treatment of a disease or disorder. The regulatory sequences driving expression from a construct will function the same in cells transduced by prior art methods as they will in cells transduced according to the invention. Support for this concept is provided by the accompanying Rule 132 Declaration of Dr. Colin M. Casimir, in paragraph 4. Specifically, Dr. Casimir states:

> "There is no reason to believe that the activity of such regulatory sequences will be different in cells transduced with a given construct according to the methods of the invention than in cells transduced with the same construct by standard means."

Therefore, assuming that the regulatory sequences on the construct are known to function in the target cell type, Applicants submit that the introduction of a construct to a quiescent cell according to the method of claim 11 will predictably result in the production of a therapeutically effective amount of a therapeutic polypeptide encoded by that construct.

Dr. Casimir also states in the Declaration that "one does not necessarily have to achieve 100% of normal levels of a transduced protein in order to treat a disease," citing several pre-filing date references in support. Therefore, where a disease or disorder involves the complete lack of or even a relatively reduced level of a given polypeptide, it is reasonable to predict that a detectable amount or an increased amount of that polypeptide expressed from cells transformed according to the invention will have therapeutic benefit. As noted in the previous response, the specification teaches, for example at page 15, lines 21-27, how to determine the level of expression of a polypeptide introduced to quiescent cells according to the claimed methods.

Dr. Casimir also states in paragraph 5 of the Declaration that "[c]ells transduced with sequences encoding therapeutic peptides have been successfully used to treat human disease." Specifically, Dr. Casimir notes the successful treatment, before September 28, 1995, of chronic granulomatous disease (see Exhibit H of the Declaration) and adenosine deaminase deficiency (Exhibits I-M). Applicants submit that the fact that the reported studies did not necessarily result in 100% correction of disease or permanent cures should not be held against the presently claimed treatment methods. Dr. Casimir states on page 3 of his Declaration that "Given the severity of the targeted diseases (untreated ADA deficiency is fatal, and CGD can be fatal), any treatment that abates the symptoms of the target disease should be considered a success."

The Examiner also expressed concern that the proliferative potential of cells transformed according to the invention would be different from the same cells that have not undergone transformation. Applicants note that neither of claims 11 or 12 requires long term proliferative capacity, and as such applicants are not required to demonstrate long term proliferative capacity. However, this concern is addressed in the Declaration of Dr. Casimir. Specifically, Dr Casimir states in paragraph 6 that:

> "The method [of the invention] of introducing a therapeutic gene sequence to a quiescent cell disclosed in the application is well-suited for the transformation of stem cells, which tend to be quiescent in vivo. Therefore, the long-term proliferative potential of the transformed cell population can actually be much greater than that of non-quiescent cells transformed according to prior art methods because stem cells by their nature have nearly unlimited capacity to proliferate."

That is, because the methods of the invention are particularly well adapted for transforming cells that have inherently greater proliferative capacity than other cell types, the long-term proliferative capacity of cells transformed according to methods of the invention can actually exceed that of cells transformed by prior art methods.

Dr. Casimir also points out in paragraph 6 of his Declaration that there are numerous reports in the prior art describing the retention of long term proliferative capacity or repopulating capacity of genetically modified stem cells. In particular, the long-term maintenance of genetically modified stem cells has been demonstrated in mice (see Exhibits G and H in the Declaration), dogs (see Exhibit I), monkeys (see Exhibits J-L) and humans (see Exhibits M-O). Dr. Casimir states that it was predictable at the time the application was filed that "stem cells transformed using the methods described in the specification would retain proliferative capacity

sufficient to permit treatment of a disease with those cells." Therefore, Applicants submit that it was predictable to one of skill in the art at the time that quiescent stem cells transformed according to the claimed methods would retain proliferative capacity sufficient to permit successful treatment of a target disease or disorder when those cells are administered to a patient.

In view of the above, Applicants submit that the operability of the invention of claim 11 as amended and claim 12 is not unpredictable and that the invention is fully enabled as claimed. Applicants therefore respectfully request the withdrawal of the §112, first paragraph rejection of these claims.

Rejection of Claims under 35 U.S.C. §102:

Claims 1, 2, 4-5 and 7-9 are rejected under 35 U.S.C. §102(e) as allegedly being anticipated by Paul et al. This rejection is maintained from the previous Office Action, in which the Examiner stated that Paul et al. teach envelope fusion vectors comprising chimeric targeting proteins that alter the host range of the vector, and contain a ligand moiety that binds to receptors present on target cells and an uptake moiety capable of promoting entry of the vector into the target cell. The Examiner stated further that Paul et al. teach packaging cells transfected with these vectors and retroviral particles produced by these cells. The Examiner also stated that the fusion protein of the Paul et al. invention can modulate the target cells in accordance with the activity of the ligand moiety (e.g. a cytokine). The Examiner also stated that ligand moieties derived from flk2 ligand can be used to direct infection to lymphohematopoietic progenitor cells. From this, the Examiner concluded that the Paul et al. reference anticipates claims 1, 2, 4-5 and 7-9. Applicants respectfully disagree.

Applicants submit that Paul et al. does not teach a method of transforming a population of quiescent cells with a nucleic acid encoding a polypeptide for treating a disease or disorder, the method comprising exposing the quiescent cells to a retroviral packaging cell line, as claimed in claim 1. In particular, the methods taught by Paul et al. do not include exposing target cells to retroviral packaging cells. All of claims 1, 2, 4, 5 and 7-9 require that the cells be transformed by exposure to a packaging cell line. All of the gene transfer methods taught by Paul et al. involve contacting retroviral particles with target cells, rather than exposing target cells to retroviral packaging cells. Therefore, the Paul et al. reference does not anticipate the invention

of claim 1, or of claims 2, 4-5 and 7-9 that depend from it. Applicant respectfully requests the withdrawal of these rejections.

Rejection of Claims under 35 U.S.C. §103:

Claim 3 is rejected under 35 U.S.C. §103(a) as allegedly being obvious over Paul et al. in view of Lyman et al. This rejection is maintained from the previous Office Action, in which the Examiner stated that Lyman et al. teaches ligands for flt3 receptors that are capable of inducing the growth, proliferation and differentiation of progenitor and stem cells, and DNA encoding flt3 ligands. The Examiner also stated that Lyman et al. asserts that the flt3 ligand of the invention is useful for disease treatment and can mobilize the number of circulating peripheral blood progenitor cells or stem cells. The Examiner also stated that Lyman et al. teaches that a cDNA encoding flt3 ligand may be transfected into cells to deliver its gene product to the targeted cell or tissue. The Examiner concluded that at the time of the invention, it would have been obvious to one of ordinary skill in the art to modify the retroviral vector of Paul (that encodes a fusion protein of a cytokine and a retroviral envelope protein) by cloning into it the flt3 ligand cDNA taught by Lyman et al., transfect the vector into cells to produce packaging cells that would produce retroviral particles capable of infecting stem cells and affect their proliferation due to the expression of the flt3 ligand. Applicants respectfully disagree.

Lyman et al. does not teach or suggest a method of transforming a population of quiescent cells with a nucleic acid encoding a polypeptide for treating a disease or disorder, the method comprising exposing the cells to a retroviral packaging cell line, as claimed in claim 1. In particular, the Lyman et al. reference does not teach or suggest the exposure of a quiescent target cell to a retroviral packaging cell line. Claim 1 and all claims dependent from it require that the cells are transformed by exposure to a packaging cell line. Therefore, Applicants submit that the Lyman et al. reference does not supply the teachings missing from the Paul et al. reference necessary in order for their combination to render obvious the invention of claim 1. Because the combination of Lyman et al. with Paul et al. does not provide all elements of the invention of claim 1, the combination does not provide all elements of the invention of claim 3, which is dependent upon claim 1. Therefore, Applicants submit that Lyman et al. and Paul et al. do not render obvious the invention of claim 3. Applicants respectfully request that the §103 rejection of claim 3 over these references be withdrawn.

Claim 6 is rejected under 35 U.S.C. §103(a) as allegedly being obvious over Paul et al. in view of Lyman et al. and further in view of Beutler et al. This rejection is maintained from the previous Office Action, in which the Examiner stated that Beutler et al. teachs a DNA encoding a chimeric protein comprising the extracellular domain of a receptor fused to IgG and a linker peptide present at a site within the chimeric protein which can be cleaved to separate the two proteins of the fusion protein. The Examiner also stated that at the time of the invention, it would have been obvious to one of ordinary skill in the art to modify the vector of Paul et al. to include sequence encoding a cleavable peptide polylinker between the sequences encoding the growth factor and the envelope protein with a reasonable expectation of success because Beutler et al. disclose the method and sequence incorporating the linker in the fusion protein. Applicants respectfully disagree.

Beutler et al. does not teach or suggest a method of transforming a population of quiescent cells with a nucleic acid encoding a polypeptide for treating a disease or disorder, the method comprising exposing the quiescent cells to a retroviral packaging cell line, as claimed in claim 1. In particular, the Beutler et al. reference does not teach or suggest the exposure of a quiescent target cell to a retroviral packaging cell line. Therefore, Applicants submit that the Beutler et al. reference does not supply the teachings missing from the Paul et al. and Lyman et al. references necessary in order for their combination to supply all elements of the invention of claim 1. Because each of these references is missing a teaching or suggestion of exposing a target quiescent cell to a retroviral packaging cell, no combination of the three references can render obvious the invention of claim 1 or any claims dependent from it. Applicants therefore submit that the invention of claim 6, which depends from claim 1, is not obvious over any combination of Paul et al., Lyman et al. and Beutler et al. Applicants therefore respectfully request that the §103 rejection of claim 6 be withdrawn.

In view of the proposed amendments to the claims and the foregoing remarks, Applicants submit that all issues raised by the Examiner in the Office Action have been addressed. Applicants therefore respectfully request the reconsideration of the claims.

Respectfully submitted,

3/19/01
Date

Mark J. FitzGerald, Ph. D.
Registration No. 45, 928 for
Kathleen M. Williams, Ph.D.
Registration No. 34,380
Attorney for Applicants
PALMER & DODGE LLP
One Beacon Street
Boston, MA 02108
Telephone: (617) 753-0451
Telecopier: (617) 227-4420

Version with markings to show changes made:

Claim 11:

11. (Amended) A method of treating a patient having a disease or disorder, the method comprising [administering to the patient an effective amount of the cells of claim 9] the steps of:

a) exposing a population of quiescent cells to a retroviral packaging cell line in vitro, said packaging cell line expressing nucleic acid encoding a growth factor so that the growth factor is displayed on the surface of the cell line, the cell line or retroviral particles carrying a vector comprising a nucleic acid encoding a polypeptide for treating said disease or disorder, wherein the surface bound growth factor induces the quiescent cells to divide, so that the nucleic acid encoding the polypeptide for treating said disease or disorder can incorporate into the genome of the cells; and

b) administering to the patient an amount of the cells of step (a) effective to treat said patient's disease or disorder.